



Investigation of bacterial contamination with *Klebsilla* and *E. coli* in the prepucal cavity of pubertal and adult age in caprine

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ABSTRACT

The herein research was carried out in order to identified the presence of bacteria inside prepuce cavity of male caprine in both mature and pubertal age with focusing on *Klebsilla* and *E coli* species. Eighty prepuce swabs (fifty form mature and thirty from pubertal age) before slaughtering and cultured on blood agar and nutrient agar, bacterial isolation were identified with biochemical teats and finally by PCR. The present study found a significant difference ($p < 0.01$) between the prepuce swabs from caprine mature age (64%) and pubertal age (40%). Six various microorganisms were detected in prepuce samples in mature age, while four types were isolated from pubertal age. Positive isolation swabs detected the presence of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus faecalis* in both ages. *Proteus mirabilis* and *Klebsiella pneumonia* was isolated from mature age only. Significant isolation ($p < 0.01$) was appear of *Escherichia coli* among all different bacterial types. This research deduce that there was that the presence of bacteria inside prepuce of male genital system in both mature and pubertal age and their where a balance between genital immunity and localization of these bacteria and any stress factor may be lead to infection with such microbes, more over the mature male had more bacterial types due to the male matting behavior, finally the *E. coli* normally found in prepuce cavity as a normal flora of both ages and the *Klebsilla* species also found in mature age as a non-specific bacterial types.

Keywords

Klebsiella, *Escherichia coli*, Prepucal cavity, Pubertal age, Adult age, Goat

Abbreviations

PCR: Polymerase Chain Reaction

Number of Figures: 0
Number of Tables: 2
Number of References: 29
Number of Pages: 6

Introduction

There is normal flora inside the body cavities without causing any diseases in the normal environment [1]. The importance of studying the normal inhabitant microflora inside the prepucal cavity lies in their role during the weakness of genital immunity due to several factors that may lead to infections with these microbes [1]. Several studies showed that a healthy genital system contains normal bacterial flora without any interference with reproductive functions [2, 3]. However, reproductive organs might get infected with unspecific bacteria that lead to decreased fertility [4]. The male is considered to be one of the causes of semen contamination [5]. The bacterial contamination of prepuce leads to penis contamination which can infect the female reproductive system during natural mating [6]. One of the complications of artificial insemination was contamination due to contact between the penis prepuce and the glans penis [1]. Some researchers stated that uterine inflammation may result from external factors entering the uterus during natural mating or incorrect artificial insemination [7]. While bacterial contamination has an adverse effect on spermatozoa fertilization ability due to its direct toxic impact on spermatozoa or indirect metabolic bacterial activity that interferes with sperm viability during semen storage. Therefore, semen is among the cofactors that spread genital infection [1]. Several factors may cause uterine infection, such as the entrance of microorganisms into the uterus cavity during mating [7], or during the usage of polluted semen [8, 9]. Glans contamination with bacteria leads to the contamination of the penis and the transfer of these bacteria into the female genital system during mating [6], indicating that the semen has a role in spreading the genital system infection of both genders as a carrier [9]. All of these factors make the idea to study the effect of age of puberty and maturity on the bacterial contamination and focusing on *Klebsilla* and *E. coli* species of the goat buck genitalia. The recent study was designed in order to detect the presence of bacteria inside prepuce cavity of male caprine in both mature as normal breeding animal and pubertal age as compensatory for aged breeding males with focusing on *Klebsilla* as non-specific bacterial types and *E coli* as a normal flora species types. Several researchers have studied infection with *E. coli* without evaluating its role in male genital organs [10; 11; 12 and 13].

Result

The Table (1) listed isolation and then identification of bacteria from mature and pubertal age of male goat. The higher percentage of isolation was detected in mature age as compared with pubertal age with a significant differences at ($p < 0.01$) (Table 1). Moreover, mature male goats (64%) and pubertal male goats (28%) were significantly ($p < 0.01$) different in terms of positive isolation percentage (Table 1). In the present study, six types of bacteria were isolated from prepuce of male goats in mature age with significant differences between the percentage of different species ($p < 0.05$) (Table 2). Four types were isolated from pubertal age with $P < 0.05$ significant differences (Table 2). A higher percentage in isolation was recorded for *E. coli* species in both mature and pubertal age 32% and 23.3%, respectively (Table 2). There was a significant difference ($p < 0.05$) between the two ages and the prevalence of this type of bacterium was significantly different ($p < 0.05$) from all other bacteria in both caprine male ages (Table 2). The next percentage was noticed in *S. aureus* species which was recorded to be 24% and 16.7% in mature and pubertal age, respectively (Table 2) with significant differences between the two ages (Table 2). *K. pneumoniae* was isolated from mature male caprine only (6%) (Table 2). *Proteus mirabilis* was also recorded in 4% of mature caprine (Table 2). *Streptococcus faecalis* was identified in 6% and 10% of mature and pubertal age, respectively (Table 2). *Pseudomonas aeruginosa* was also isolated from 4% of mature-age and 6.7% of pubertal-age goats (Table 2). *S. faecalis* and *P. aeruginosa* also showed significant differences ($p < 0.05$) between the two ages (Table 2).

Table 1. The number of prepuce samples, positive and negative isolation in puberty and mature male goat

Animal age	Samples positive isolation		Samples negative isolation		Significance
	number	Percentage	number	Percentage	
Mature	32	64%	18	36%	*
Puberty	12	40%	18	60%	*
Significance		*		*	

* Significant differences at $p < 0.01$.

Table 2. Types of isolated bacteria from cervical samples during different estrus phases of slaughtering cows.

Types of isolated bacteria	Mature males		Puberty males		Significance
	Positive	Percentage	Positive	Percentage	
<i>Escherichia coli</i>	16	32%	7	23.3%	*
<i>Klebsiella pneumoniae</i>	3	6%	-	0%	*
<i>Pseudomonas aeruginosa</i>	2	4%	2	6.7%	*
<i>Staphylococcus aureus</i>	12	24%	5	16.7%	*
<i>Proteus mirabilis</i>	2	4%	-	0%	*
<i>Streptococcus faecalis</i>	3	6%	3	10%	*
Total isolation	38	*	15	*	

Some of these swabs contain more than one bacterium so the number of total will be more than positive isolation numbers.

* Significant differences between percentage at $p < 0.01$.

Discussion

This herein data showed that the external parts of male genital organs of goats had contamination with many various bacteria; our result become agrees in this part with others workers [6]. This study indicated that there were 64% of mature male goat had positive bacterial prepuce swabs as compared with 28% of pubertal male. This fact indicated that the male genital system contain normal microflora had no effect on reproductive activity. This is agreeing with other studies [2; 3 and 4]. Some authors said that the isolation of bacteria from male and the female was symmetric [14]. This recent study is similar to this statement. Normal flora bacterial types could be activated during stress factors and it causes diseases as a pathogenic type [15]. This part indicated the importance of studying the contents of bacteria before using of male for breeding. This study isolated six types of bacteria represent the high percentage of it the localization of *Escherichia coli* and *Staphylococcus aureus* in mature age, whereas four types were isolated in pubertal age. This is lower than that which isolated from Al-Delemi *et al.* [1], and higher than Zaid *et al.* [16] in male goat. Zaid and Al-Zubaidy [15] claimed that there was relationship between bacterial number and bacterial types and fertilization of sperms; while Marinov *et al.* [17] stated that the 2nd ejaculation had little bacterial number than 1st one. The using of artificial techniques will be decrease uterine infections [18]. Bacterial contamination plays an important harmful role on uterus cavity especially the uterine glands [19]. Natural service considered as an important factors of uterine infection [20]. The response of endometrium against inflammation was controls by antigens and phys-

iological events [7], it was appear within 0.5-1 hour after matting [15] this response is important to clear uterine cavity from bacteria and dead spermatozoa [16]. Lateness of uterine elimination from bacteria, fluid and debris after matting may results from many causes as myometrial activity decrease or myometrial activity duration will changed or myometrial activity frequency interacted [21], endometrium changed of vessels [15], altered hormonal response [21] mucus discharge stopping [22].

The result of identification of *Klebsiella pneumoniae* partially agrees with Aziz *et al.* [2] who said the importance of this microorganism in fertility. The lower percentage of isolation of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Streptococcus faecalis* due to it was considered as non-specific bacterium in genital system, so this may be the causative of lower isolation of those bacteria in pubertal age which related to decreases of natural mating with female than mature male. This type of bacteria is isolated in our result. This is fit with the result of Al-Delemi *et al.* [1]. The recent work detected that the possibility of make mixed bacterial isolation from the same swabs, this is agree with Al-Delemi [5].

The *E. coli* isolated by Al-Delemi [5] after matting and describes it as a normal flora inside uterine cavity, its origin from digestive cavity and may transported into urogenital tract of the female [20]. This isolation of such bacteria is done in the recent study. Al-Zubaidi *et al.* [23] stated that the *E. coli* caused vaginitis and the intravaginal sponge do not change microflora of vagina. *Escherichia coli* and *Pseudomonas aeruginosa* maybe result during matting from semen contamination with feces [15], or it came from female after mating due to its origin from female genital sys-

tem [1]. This is the origin of the bacterial types in our study. The release of *Staphylococcus aureus* in normal male semen with large number [16], also it had no pathological effect although of its high release [15], so it can easily identify in high level before or after matting. This may be explaining the higher percentage of isolation of such bacteria in our study. Al-Badry *et al.*, [6] stated that this type of bacterium largely release with semen of ruminants without any clinical infections. The isolation of *Proteus mirabilis* was firstly done in this study from external part of genital organs; this indicated the existence of such bacteria flora in male genitalia. The bacterium may cause inflammation of female genital tract [24] in the she-camel. The increase of bacterial isolation may be come from dam during pubertal periods that appear again during maturation period [15]. This type of bacteria needs to be studied more for its relation with genital infection of both male and female. The isolation of *Pseudomonas aeruginosa* after matting in the herein study was previously detected by Al-Delemi [5] as a normal flora in ruminants. *Pseudomonas aeruginosa* had no damage effect to the genital tract [2]. This bacteria is isolated in the herein study. The presence of this bacterium inside semen results in a low fertility in male, and there was a high correlation with sterility [16]. The isolation of *Streptococcus faecalis* in high percentage in our study may come from infertility or abortion of the dam [15]. The limitation of our study its need to study the effect of matting behavior and female genital effect on bacterial contamination of external part of male.

From above we concluded that the presence of bacteria inside prepuce of male genital system in both mature and pubertal age. There were a balance between genital immunity and localization of these bacteria and any stress factor may be lead to infection with these microbes. Moreover, the mature male had more bacterial types due to the male matting behavior. Finally the *E. coli* normally found as a normal flora in prepuce cavity of both ages and the *Klebsilla* species also founded as a non-specific bacterial type.

Materials and Methods

Animals and sample collection

Eighty swabs were taken from the prepuce of healthy male goats before slaughtering consisting of 50 samples from mature males (around 3 years) and 30 samples from puberty males (below 1 year). These swabs were taken from the prepuce cavity after disinfecting the prepuce orifice with ethyl alcohol (70%, France). Cotton swabs (Amies transport medium, China) were inserted inside the prepuce cavity and moved several times. Then, these samples were transported to a private laboratory.

Isolation of bacteria

Media used to isolate the bacteria included sheep blood agar,

MacConkey agar, nutrient agar, eosin methylene blue agar, and brain heart infusion agar. The media were incubated under aerobic conditions at 37°C for 24 hours. This was done according to the “Bergey’s Manual of Systematic Bacteriology” [25].

Bacterial Identification

For all types of bacteria, identification was completed according to the “Bergey’s Manual of Systematic Bacteriology” [26] by culturing, biochemical tests, and morphological characteristics. The observed characteristics of colonies on agar surfaces include color, size, consistency, pigment production, and shape. Cellular morphology was assessed by gram stain under a microscope. The biochemical tests included catalase, oxidase, IMViC, TSI, coagulase, urease production, gelatin liquefaction, and hemolysis.

Identification by PCR

PCR (PerciGenome, USA) was used to amplify *Escherichia* genus-specific gene 16S rRNA of *E. coli*. Primer pairs were used to identify the gene (F 5’-GACCTCGGTTTAGTTCACAGA-3’ and R 5’-CACACGCTGACGCTGACCA-3’). A total of 20 µl reaction mixture consisted of 3 µl genomic DNA, 10 µl PCR master mix (Promega, USA), and 1 µl of two primers. The final volume was adjusted to 20 µl with 5 µl of nuclease-free water. Initial denaturation was at 95°C for 5 min, denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec, and extension at 72°C for 60 sec. The final extension was completed at 72°C for 5 min, and the reaction was performed in 30 cycles. Electrophoresis (Cleaver, UK) was conducted in 2% agarose gel at 100 v for 30 min. Staining was performed with ethidium bromide under a UV trans-illuminator according to Schippa *et al.* [27]. For *K. pneumonia*, the detection was performed by inf B1 gene using the primers (F 5’-CTC-GCTGCTGGACTATAT TCG-`3 and R 5’-CGCTTTCAGCT-CAAGAACTTC-`3). A reaction mixture of 25 µl contained 2 µl DNA, 1 µl of two primers, 12.5 µl master mix, and 8.5 µl nuclease-free water. Initial denaturation was at 95°C for 5 min, 30 cycles at 95°C for 0.5 min, 55°C for 30 sec, 72°C for 0.5 min, and final extension at 72°C for 7 min. The PCR product was analyzed (PerciGenome USA) using gel electrophoresis (Cleaver UK) 1% agarose, was stained with ethidium bromide, and visualized with a UV illuminator. This was completed according to Abd Alwahed *et al.* [28].

Statistical analysis

Statistical analysis was performed using the Chi-square test to detect the variation between the percentages of groups at *p* < 0.01 and *p* < 0.05. This was performed using SAS [29].

Authors' Contributions

Ansam Khalid Mohammed conceived and planned the experiments and carried out the experiments with planned and carried out the simulations and contributed to sample preparation and contributed to the interpretation of the results and took the lead in writing the manuscript. The author provided critical feedback and helped shape the research, analysis and manuscript.

Acknowledgements

We would like to thank the privet workers that take care of animals of the study.

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Competing Interests

The authors declare that there is no conflict of interest.

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Khalid Mohammed , IJVST 2023; Vol.15, No.3 DOI: 10.22067/ijvst.2023.82390.1249

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How to cite this article

Khalid Mohammed A. Investigation of Bacterial Contamination with Klebsiella and Escherichia coli in the Prepuccal Cavity of Pubertal and Adult Caprine. Iran J Vet Sci Technol. 2023; 15(3): 27-32.
DOI: <https://doi.org/10.22067/ijvst.2023.82390.1249>
URL: https://ijvst.um.ac.ir/article_44315.html